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1. Your reference — 45072.GB01/SG/AA

2. Patent application number *(The Patent Office will fill in this part)* — 0221339.5
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3. Full name, address and postcode of the or of each applicant *(underline all surnames)*

CSEM Centre Suisse d'Electronique
et de Microtechnique SA
Jaquet-Droz 1
CH-2007 Neuchâtel
Switzerland

Patents ADP number *(if you know it)* 08062101001

If the applicant is a corporate body, give the country/state of incorporation — Switzerland

4. Title of the invention — Fluidic System

5. Full name, address and postcode in the United Kingdom to which all correspondence relating to this form and translation should be sent

Reddie & Grose
16 Theobalds Road
LONDON
WC1X 8PL

Patents ADP number *(if you know it)* — 91001 ✓

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Country	Priority application *(If you know it)*	Date of filing *(day/month/year)*

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Number of earlier application	Date of filing *(day/month/year)*

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   a) any applicant named in part 3 is not an inventor, or
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Continuation sheets of this form	
Description	9
Claim(s)	6
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Priority documents	
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Statement of inventorship and right to grant of a patent *(Patents Form 7/77)*	
Request for preliminary examination and search *(Patents Form 9/77)*	1
Request for substantive examination *(Patents Form 10/77)*	
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11. I/We request the grant of a patent on the basis of this application.

Signature Reddie & Grose Date 11September 2002

Reddie & Grose

12. Name and daytime telephone number of person to contact in the United Kingdom

S J N GOODMAN
01223 360350

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Fluidic System

The invention relates to a fluidic system for analysing biomolecules in solution and to a method of analysing biomolecules in solution.

The invention is applicable to materials and methods for the analysis of biomolecules, such as antibodies, antigens, enzymes, and proteins, in fluid samples using solid-phase assays. The invention has particular utility when performing analyses using packed microbeads.

Microparticles in the form of microbeads can be made of a variety of materials, such as glass, polystyrene, or other polymers and when utilized as solid phase assays are coated with the appropriate ligand for binding the molecular species to be analyzed.

Flowing a sample containing the molecular species of interest, called analyte hereafter, through a bed of microbeads speeds the reactions between the analyte and the ligand immobilized on the surfaces of the microbeads. The increased reactive surface area, the reduced diffusion distance, and the stirring of the sample due to the turbulent flow within the bed of beads cause this enhancement in reactivity. The immediate advantages are a higher sensitivity, a shorter analysis time, and a reduced consumption of analyte and reagents. The use of such packed microbeads in microfluidic systems enhances these advantages even further.

Separation and concentration of biomolecules such as proteins, chromosomes, nucleic acids, and the like is important in various detection, isolation, and quantification tests in biochemistry and diagnostics. Specifity, sensitivity, and time are the important parameters in the separation and concentration schemes. Furthermore, a low consumption of sample and reagents makes tests less invasive for a patient and cheaper, respectively.

Microfluidic systems require only small amounts of sample and reagents and the small volumes can be handled with better precision than in conventional macroscopic systems, reducing the costs and error rates of analysis. The high surface to volume ratio in microfluidic systems speeds reactions and creates conditions more relevant to biological ones. To enable analyte-ligand tests within such systems, the ligand has to be brought into the system either by directly functionalizing defined parts of the microfluidic channel walls or by introducing and retaining functionalized microbeads. The latter option not only allows one to use microbeads which can be functionalized by standard techniques in large quantities outside the microfluidic system, but also enhances the sensitivity and reaction speed because of the sieve-like function of a packed bed of microbeads within a microfluidic channel.

WO 01/34302 "Biochannel Assay for Hybridization with Biomaterial" describes the use of microchannels that have separated regions with specific ligands bound to porous polymer, beads or structures fabricated into the microchannels to function as a solid phase assay, but does not describe how beads can be introduced and retained in the microchannels. Retaining the microbeads in the microchannels can be a difficult task.

US 6,120,734 "Assay System", WO 00/50172 "Manipulation of Microparticles in Microfluidic Systems", and Oleschuk et al., Trapping of Bead-Based Reagents within Microfluidic Systems: On-Chip Solid-Phase Extraction and Electrochromatography, Analytical Chemistry 2000, 72, 585-590 describe the use of microparticles in microfluidic systems as solid-phase assays and employ physical barriers for bead retention. Such physical barriers for microbeads are difficult to fabricate, can be applied to only a certain size range of beads, and the beads cannot easily be removed or further manipulated.

Another method for bead retention is the use of magnetic

forces (Fan et al., Dynamic DNA Hybridization on a Chip Using Paramagnetic Beads, Analytical Chemistry, 1999, 71, 4851-4859; US 5,972,721 "Immunomagnetic Assay System for Clinical Diagnosis and other Purposes"). However, this method requires special microbeads with magnetic properties and bulky sources for generating the magnetic fields, which are difficult to integrate into microfluidic systems.

The invention provides a fluidic system for analysing biomolecules in solution comprising an inlet port, an outlet port, a set of interdigitated electrodes extending across the channel, means for moving liquid through the fluidic system, means for flowing a suspension of a given type of microparticles through the fluidic system, means for applying an AC voltage having an appropriate frequency for retaining a given type of microparticles in the region of the electrodes by means of dielectrophoresis, the microparticles being functionalized with appropriate ligand molecules, and means for flowing a sample liquid containing the analyte specifically bound by the ligand molecules on the microparticles through the fluidic system, thereby perfusing the retained microparticles.

Various preferred, advantageous, and/or alternative features of the invention are set out in the dependent claims to which reference should now be made.

Dielectrophoresis is a method where a force can be applied to dielectric particles in order to manipulate them. This force is caused by an electric field, which can be generated by an AC-voltage applied to microelectrodes. Particles will either be attracted to or repelled from the microelectrodes depending on the dielectric properties of the particles and their surrounding medium and the frequency of the applied voltage (see for example Pohl, Dielectrophoresis, Cambridge University Press, Cambridge, 1978) For a given set of particles and suspending medium, the magnitude and direction of the dielectrophoretic force can be tuned with the frequency of the applied voltage, allowing one to choose and

separate specific particle types from a mixed suspension.

WO 02/31179 "Multiplex Assays using Nanoparticles" describes using a microfluidic device with microelectrodes to separates nanoparticles by dielectrophoresis, after the nanoparticles have bound analyte molecules by specific interaction. The device uses the change in dielectric properties upon analyte binding to detect the presence of said analyte, but does not generate a packed bed of beads to function as a solid phase assay.

US 6,352,838 "Microfluidic DNA Sample Preparation Method and Device" describes using dielectrophoresis for capturing target material within a microdevice, said target material being DNA, spores, bacteria or polystyrene beads. It does not describe capturing microbeads by dielectrophoresis and subsequently perfusing them with sample containing the analyte.

Besides the general advantages of microfluidic systems using microbeads mentioned above, a system according to the invention has the following advantages:

a) preconcentration of beads is possible in microchannels without physical barriers or bulky magnetic field generating apparatus;

b) once an assay has been finished, the used beads can be removed from the microchannels and fresh beads may be brought in, that is the device can be reused;

c) the system is versatile, because the actual test performed can be chosen by the introduction of microbeads functionalized with the appropriate ligand. The microchannels of the microfluidic system remain the same, reducing production costs for such systems; and

d) several bead retaining sites can be formed within the microchannels by successive activation of dielectrophoresis areas, creating the possibility of multistep analysis and multistep analysis on a single

device

The present invention provides a microfluidic system capable of retaining and concentrating microbeads in defined locations within the microfluidic channels, thereby creating a packed bed of microbeads. Retaining and accumulation of the microbeads may be accomplished without any physical barriers by integration of microelectrodes producing dielectrophoresis into the microchannels. By choosing the appropriate voltage and frequency applied to the microelectrodes, the dielectrophoretic retaining force can be tuned to retain only microbeads with specific dielectric properties. Subsequently, the retained microbeads can be perfused with liquids containing analytes, reagents, rinsing buffers, etc. If the microbeads are functionalized with molecules specific to a given analyte, such a system can act as a micro-assay for the given analyte. Detection of the analyte can be done at the bead retention site by any convenient techniques, for example fluorescence. After analyte detection has been completed, the beads can easily be removed from the microfluidic system by switching off the voltage applied to the microelectrodes and rinsing the microchannels with a buffer solution.

In an alternative embodiment of the invention, the microbeads can be released from the retaining electrodes after analyte accumulation for analyte detection at a different bead retaining location within the microfluidic system. This would, for example allow for a further concentration of beads in a smaller area, thus simplifying optical detection techniques, or enable analysis of individual beads by cytometry.

The invention further provides a method for analysis of biomolecules in solution comprising the steps of:

a) providing a fluidic system having an inlet and outlet port and containing a set of interdigitated electrodes and a means of moving liquid through the fluidic system;

b) applying an AC voltage to said electrodes with an appropriate frequency for retaining in the region of the electrodes a given type of microparticles which are functionalized with appropriate ligand molecules;

c) flowing a suspension of said type of microparticles through the fluidic system and retaining the microparticles at the interdigitated electrodes by means of dielectrophoresis;

d) flowing a sample liquid containing the analyte specifically bound by the ligand molecules on the microparticles through the fluidic system, thereby perfusing the retained microparticles; and

e) detecting the presence of analyte bound to the microparticles.

f) A method as claimed in Claim 12 in which in step c) a plurality of types of microparticle having different dielectric properties are flowed through the fluidic system and the type of microparticle specified by the choice of frequency in step b)is retained at the interdigitated electrodes by means of dielctrophoresis.

Various preferred, advantageous, and/or alternative features of the method according to the invention are set out in the dependent claims to which reference should now be made.

The above and other features and advantages of the invention will be apparent from the following description, by way of example, of an embodiment of the invention with reference to the accompanying drawings, in which:

Figure 1 shows in block schematic form a system for for analysing biomolecules in solution according to the invention, and

Figure 2 shows in greater detail a microchannel, input and output ports, and interdigitated electrodes forming part of the system of Figure 1.

As shown in Figure 1 the system comprises a plurality of reservoirs 11-1,11-2,...11-n for containing microbeads, reagents, buffers,samples,etc. A pump 12, which may be of any convenient form, but is typically a syringe pump is used to introduce the appropriate materials into a channel 13. The channel 13 contains an interdigitated set of electrodes 14 which have an AC voltage applied across them by means of an AC voltage generator 15. A drain 16 collects the waste material after it has passed through the channel 13. A detector 17 is provided to detect the analyte on the beads at the site of the electrodes. Thus Figure 1 shows of a microfluidic system containing one or more dielectrophoretic retention sites for microparticles.

Figure 2 shows part of the microfluidic system in its simplest form of a single microchannel 21 having with an inlet port 22 and outlet port 23 and containing a set of interdigitated microelectrodes 24, which can be powered with an AC voltage of 0-20 V and 100 Hz to 20 MHz. As

shown in Figure 2a fluid flow 25 through the microchannel 21 can be used to introduce microbeads 26, which have been functionalized with appropriate ligand molecules. The diameter of the beads may be chosen to be within the range of 100 nm to 10 mm. If as shown in figure 2b, the microelectrodes 24 are powered with the appropriate voltage and frequency for retaining the functionalized microbeads 26, the microbeads 26 will form a packed bed 27, which will function as a solid-phase microbead array. This packed bed 27 can subsequently be perfused with a sample containing the analyte 28 specifically bound by the ligand immobilized on the microbeads. As shown in Figure 2c the analyte will be separated and concentrated by the retained beads 29 and can be detected directly or indirectly by further perfusion of labeled reagent molecules.

In a non-limiting example embodiment of the invention, the microfluidic system consists of the single microchannel 21 between 100 mm and 4 mm wide, less than 30 mm high, and 10 mm long. Interdigitated electrodes 24 have 10 mm width and 10 mm spacing and span the entire length of the microchannel. Fluid is pumped through the microchannel with a syringe pump 12 generating a flow rate of up to 10 mm/s. A 2.5% suspension of streptavin labeled 2 mm polystyrene beads is diluted at a ratio of at least 1:10 in an aqueous buffer solution with a conductivity of less than 1000 mS/m, called working solution hereafter. If a voltage of 16 V and at 100 kHz is applied to the interdigitated electrodes, the 2 mm beads will be retained at the electrodes by positive dielectrophoresis, thus forming a packed bed of microbeads. While keeping the voltage applied to

the interdigitated electrodes, the bead bed can first be rinsed by flowing working solution through the microchannel to remove unbound microbeads and secondly be perfused with the analyte by flowing fluorescein labeled biotin solved in the working solution through the microchannel. The microchannel can then be rinsed with pure working solution to remove excess biotin molecules. Finally, the amount of bound biotin can be detected with a fluorescence light microscope.

This example is intended to illustrate the functionality of the present invention and not to limit it in spirit or scope. The system may be operated with any analyte-ligand system.

Claims

1. A fluidic system for analysing biomolecules in solution comprising an inlet port, an outlet port, a set of interdigitated electrodes extending across the channel, means for moving liquid through the fluidic system, means for flowing a suspension of a given type of microparticles through the fluidic system, means for applying an AC voltage having an appropriate frequency for retaining a given type of microparticles in the region of the electrodes by means of dielectrophoresis, the microparticles being functionalized with appropriate ligand molecules, and means for flowing a sample liquid containing the analyte specifically bound by the ligand molecules on the microparticles through the fluidic system, thereby perfusing the retained microparticles.

2. A system as claimed in Claim 1 comprising means for flowing a plurality of types of microparticles with different dielectric properties through the fluidic system, and means for applying different frequency voltages to the electrodes to retain selected ones of the types of microparticles.

3. A system as claimed in Claim 1 or Claim 2 comprising means for detecting the presence of the analyte bound to the microparticles at the retention site of the microparticles.

4. A system as claimed in any of Claims 1 to 3 comprising means for flowing a solution containing reagent molecules specific to the analyte molecules through the fluidic system to perfuse the analyte bound by the ligand molecules on the microparticles.

5. A system as claimed in Claim 4 in which the presence of reagent molecules bound to the microparticles is detected at the retention site of the microparticles.

6. A system as claimed in any preceding claim comprising means for flowing a rinsing liquid through the fluidic system to remove unbound microparticles and analyte molecules, respectively.

7. A system as claimed in any preceding claim comprising means for removing the AC voltage from the interdigitated electrodes to release the microparticles and means for detecting the presence of analyte bound to the microparticles at a site separate from the interdigitated electrodes.

8. A system as claimed in any preceding claim in which the fluidic system comprises a glass or silicon support with microstructured microelectrodes and a PMMA, PDMS, or other polymer cover with structured microchannels and an inlet and outlet port.

9. A system as claimed in any preceding claim in which the microparticles consist of polystyrene microbeads with diameters between 100 nm and 10 mm.

10. A system as claimed in any preceding claim in which the fluid flow is generated by a syringe pump.

11. A fluidic system for analysing biomolecules in solution substantially as described herein with reference to the accompanying drawings.

12. A method for analysis of biomolecules in solution comprising the steps of:

a) providing a fluidic system having an inlet and outlet port and containing a set of interdigitated electrodes and a means of moving liquid through the fluidic system;

b) applying an AC voltage to said electrodes with an appropriate frequency for retaining in the region of the electrodes a given type of microparticles which are functionalized with appropriate ligand molecules;

c) flowing a suspension of said type of microparticles through the fluidic system and retaining the microparticles at the interdigitated electrodes by means of dielectrophoresis;

d) flowing a sample liquid containing the analyte specifically bound by the ligand molecules on the microparticles through the fluidic system, thereby perfusing the retained microparticles; and

e) detecting the presence of analyte bound to the microparticles.

f) A method as claimed in Claim 12 in which in step c) a plurality of types of microparticle having different dielectric properties are flowed through the fluidic system and the type of microparticle specified by the choice of frequency in step b)is retained at the interdigitated electrodes by means of dielctrophoresis.

13. A method according to claim 12 in which the presence of the analyte bound to the microparticles is detected at the retention site of the microparticles.

14. A method according to claim12 or Claim 13 in which after step d) a solution containing reagent molecules, specific to the analyte molecules, is flowed through the fluidic system, thereby perfusing the analyte bound by the ligand molecules on the microparticles.

15. A method according to claim 14 in which the presence of reagent molecules bound to the microparticles is detected at the retention site of the microparticles.

16. A method according to any of claims 12 to 15 in which after steps c) and d) a rinsing liquid is flowed through the fluidic system to remove unbound microparticles and analyte molecules, respectively.

17. A method according to claim 16 in which the presence of the analyte bound to the microparticles is detected at the retention site of the microparticles.

18. A method according to claim 16 in which after the rinsing after step d) a solution containing reagent molecules, specific to the analyte molecules, is flowed through the fluidic system, thereby perfusing the analyte bound by the ligand molecules on the microparticles and after this step, a rinsing liquid is flowed through the fluidic system for removing unbound reagent molecules.

19. A method according to claim 18 in which the presence of reagent molecules is detected at the retention site of the microparticles.

20. A method according to any of claims 12 to 19 in which the microparticles are released after analyte binding by removing the AC voltage from the interdigitated electrodes and in which the presence of analyte bound to the microparticles is detected at a separate site within the fluidic system.

21. A method according to any of claims 12 to 19 in which the microparticles are released after analyte binding by removing the AC voltage to the interdigitated electrodes and in which the presence of analyte bound to the microparticles is detected outside the fluidic system.

22. A method according to claim 4, where the microparticles are released after reagent binding by no longer applying the AC voltage to the interdigitated electrodes and where the presence of reagent bound to

the microparticles is detected at a different site within the fluidic system.

23. A method according to claim 14 in which the microparticles are released after reagent binding by no longer applying the AC voltage to the interdigitated electrodes and where the presence of reagent bound to the microparticles is detected outside the fluidic system.

24. A method according to claim 16 in which the microparticles are released after rinsing by removing the AC voltage to the interdigitated electrodes and in which the presence of analyte bound to the microparticles is detected at a separate site within the fluidic system.

25. A method according to claim 16 in which the microparticles are released after rinsing by removing the AC voltage to the interdigitated electrodes and in which the presence of analyte bound to the microparticles is detected outside the fluidic system.

26. A method according to claim 18 in which the microparticles are released after rinsing by removing the AC voltage from the interdigitated electrodes and in which the presence of analyte bound to the microparticles is detected at a separate site within the fluidic system.

27. A method according to claim 18 in which the microparticles are released after rinsing by removing the AC voltage from the interdigitated electrodes and in which the presence of analyte bound to the microparticles is detected outside the fluidic system.

28. A method according to any of claims 12 to27 in which the fluidic system constructed with structured microchannels and the inlet and outlet port and with

microstructured microelectrodes from a glass or silicon support and a PMMA, PDMS, or other polymer cover.

29. A method according toclaim 28 in which the interdigitated microelectrodes span the whole width of the fluidic channel, have a width between 1 and 20 mm and a gap between the electrodes between 1 and 20 mm.

30. A method according to any of claims 12 to29 in which the microparticles consist of polystyrene microbeads with diameters between 100 nm and 10 mm.

31. A method according to any of claims 12 to 30 in which the fluid flow is generated by a syringe pump, the ligand bound to the microbeads is streptavidin and the analyte contained in the sample liquid is fluorescein labeled biotin, and in which the detection of fluorescein labeled biotin bound to the microbeads functionalized with streptavidin is carried out using a fluorescence microscope.

32. A method for analysis of biomolecules in solution substantially as described herein with reference to the accompanying drawings.

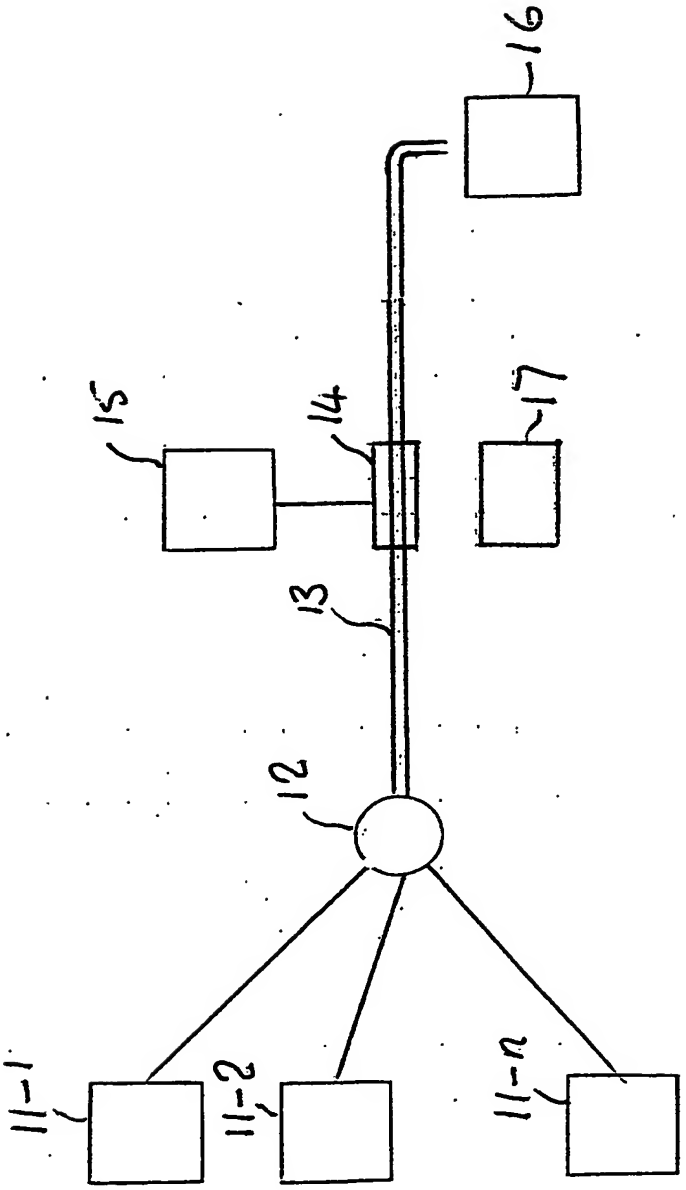
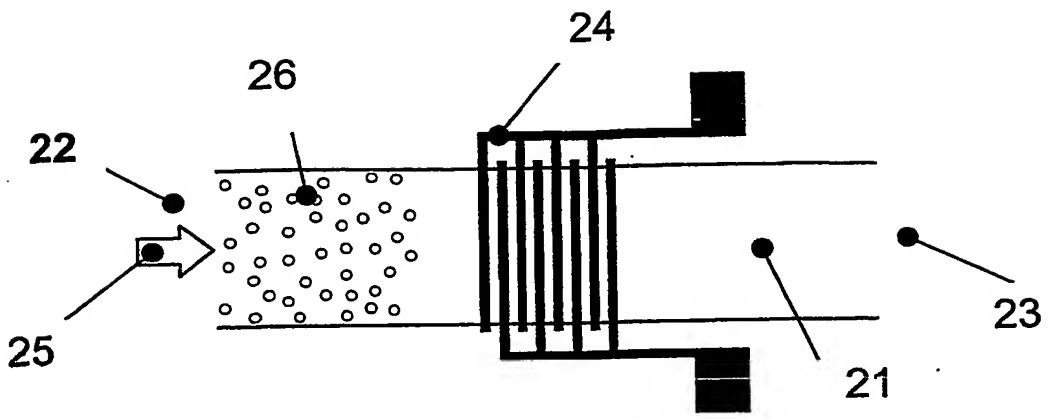
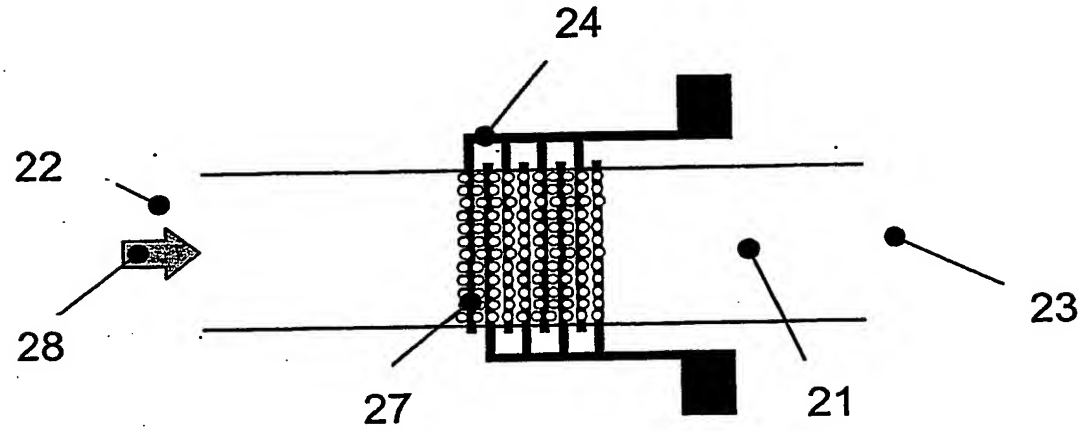


FIGURE 1.





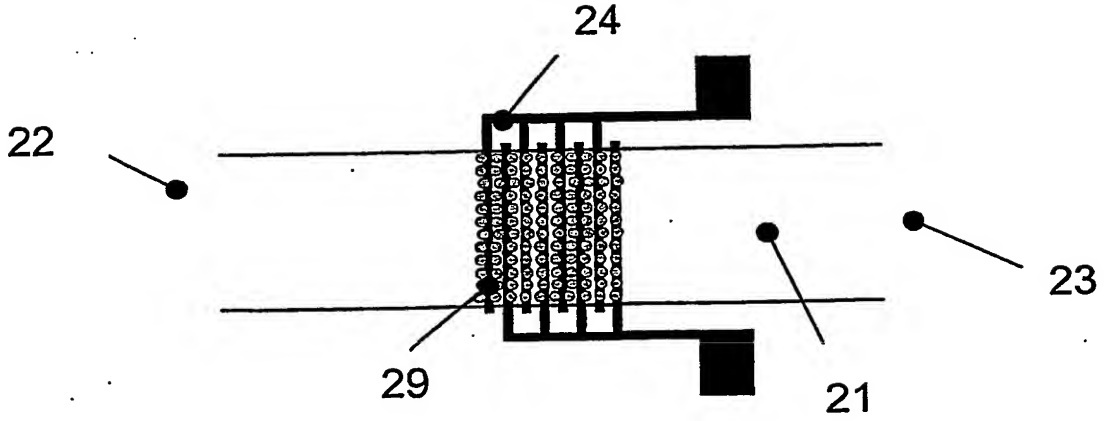


Figure 2